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Contents of Anthocyanins and Ellagitannins in Selected Foods Consumed in Finland

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Numerous in vitro and in vivo studies have suggested that dietary anthocyanins and ellagitannins or ellagic acid might have beneficial health effects. Epidemiological evidence on the disease-preventing potential of these polyphenols is lacking, due to the absence of reliable data on their contents in foods. In this study was analyzed the content of anthocyanins and ellagitannins (as ellagic acid equivalents after acid hydrolysis) in foods consumed in Finland, including berries, fruits, vegetables, and processed products, using high-performance liquid chromatographic (HPLC) methods. Anthocyanins were detected in 41 of 54 selected food items. The total anthocyanin content varied in berries from 1 to 611 mg/100 g, in fruits from 2 to 66 mg/100 g, and in vegetables from 3 to 75 mg/100 g of fresh weight as the weight of the aglycone. Ellagitannins were screened in 33 food items, but were detected only in 5 species of berries, that is, in cloudberry, raspberry, rose hip, strawberry, and sea buckthorn, the content ranging from 1 to 330 mg/100 g. The results underscore the superiority of berries, especially dark blue or red berries, as excellent sources of anthocyanins and certain berries of the Rosaceae family as the major source of ellagitannins in the Finnish diet.

KEYWORDS: Anthocyanin; ellagitannin; ellagic acid; database; polyphenol; berries; fruits; vegetables

INTRODUCTION

Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins, which are polyhydroxyl and polymethoxyl derivatives of 2-phenylbenzopyrylium (flavylium cation), and belong to the flavonoid class of polyphenols (*1*). In nature, anthocyanidins are mostly found as (acyl)glycosides, and free aglycones rarely occur in fresh plant material. The most widespread anthocyanidins present in higher plants are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (**Figure 1A**). Glucose, galactose, rhamnose, arabinose, rutinose (rhamnosylglucose), sophorose (glucosylglucose), and sambubiose (xylosylglucose) are the most common sugar moieties, usually linked to positions 3 and 5 of the anthocyanidin skeleton, and may be substituted by aliphatic, hydroxybenzoic, or hydroxycinnamic acids.

Anthocyanins are mostly responsible for the red, blue, and purple colors of flowers, fruits, and vegetables. Dietary sources include red and blue berries, fruits and vegetables with colored skin, and red wine (*1*). The daily consumption of anthocyanins was recently estimated to be 12.5 mg/day/person in the United States, with fruits and berries providing 70% of the total intake (*2*). Estimation of the daily intake of anthocyanins in different countries and populations is still difficult and inaccurate, because of the incomplete data on the anthocyanin quantities in foods and the composition and intake of consumed foods.

Ellagitannins (**Figure 1B**), which belong to the hydrolyzable tannin class of polyphenols, are complex derivatives of ellagic acid (*3*, *4*). They contain one or more hexahydroxydiphenic acid (HHDP) moieties esterified to a polyol, usually glucose. Hydrolysis of ellagitannins with acids or bases yields HHDP, which spontaneously lactonizes to ellagic acid (**Figure 1C**). This reaction has been utilized for the detection and quantification of ellagitannins as ellagic acid equivalents after acid hydrolysis of food samples (*5*−*7*). Also, in the human gastrointestinal tract, ingested dietary ellagitannins are hydrolyzed to release ellagic acid, which is further metabolized by the colonic microflora to yield bioavailable urolithins A and B (*8*−*10*). The occurrence of ellagitannins in common foods is limited to a few berry, fruit, and nut species. They have been detected in berries of the genus *Rubus* (raspberry, blackberry, cloudberry, arctic bramble) and the genus *Fragaria* (strawberry), pomegranate, walnuts, and some other nuts, and oak-aged wines (*3*, *5*, *11*). In addition to polymeric ellagitannins, minor amounts of ellagic acid are usually present as free and glycosylated forms (*12*−*14*).

Like other polyphenols, anthocyanins and ellagitannins/ellagic acid possess a wide range of biological activities, which suggest that they could have beneficial effects on human health. Their antioxidant and free radical scavenging, antimicrobial, anti-

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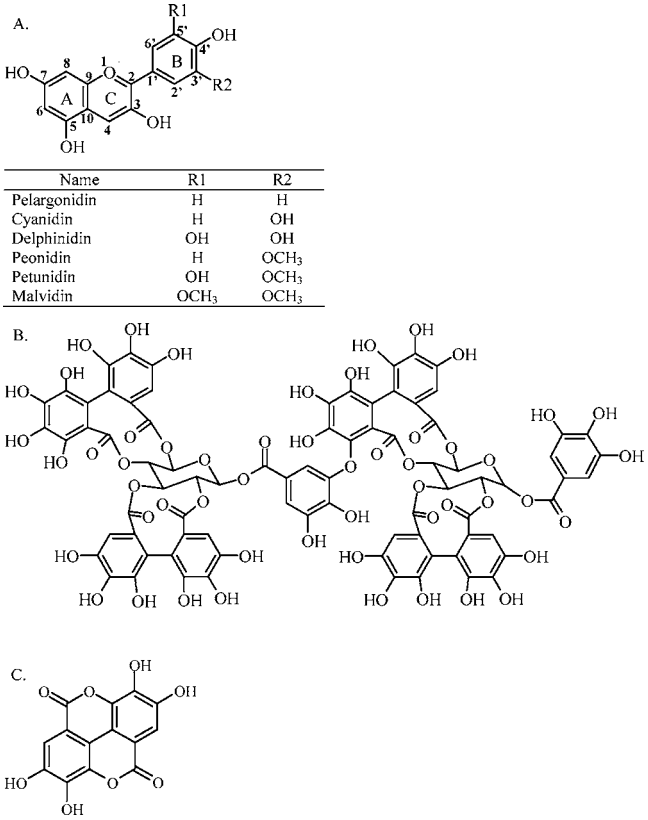

Name	R1	R2
Pelargonidin	H	H
Cyanidin	H	OH
Delphinidin	OH	OH
Peonidin	H	OCH_3
Petunidin	OH	OCH_3
Malvidin	OCH_3	OCH_3

Figure 1. Chemical structures of anthocyanidins (**A**), ellagitannin (**B**), and ellagic acid (**C**).

inflammatory, antimutagenic, and anticarcinogenic properties have been extensively reviewed (*1*, *3*, *15*−*20*). Epidemiological evidence indicates that dietary polyphenols may be protective against chronic diseases (*21*). However, these studies have included only flavonols, flavones, flavanones, and catechins, because comprehensive data on the contents of many other polyphenols in foods are lacking. The aim of the present study was to determine the contents of anthocyanins and ellagitannins in berries, fruits, vegetables, and processed products consumed in Finland. The analyses were performed during the years 2003−2006, as part of a larger study of the contents of various polyphenols (phenolic acids, anthocyanins and other flavonoids, proanthocyanidins, and ellagitannins) in foods consumed in Finland (*22*−*24*).

MATERIALS AND METHODS

Samples. The sampling was performed as previously described (*23*, *24*). Almost all berries available and fruits, vegetables, and beverages commonly consumed in Finland were selected for the study (**Table 1**). All samples, except fresh berries, cherries, rhubarbs, and red wine, were purchased from retail stores in the Helsinki, Kuopio, and Forssa areas during 2003−2005. All three of the major food chains were represented. Berries (all domestic) were purchased from market stalls and one wholesaler in the Kuopio area during the summers of 2003−2005. Red wine samples were purchased from Alko Inc. in Forssa in the fall of 2004 (Alko Inc. has a monopoly in Finland over retail sales of beverages containing over 4.7% alcohol by volume).

One pooled sample was prepared representing 9−12 subsamples of each food item, except for berries, for which the number of subsamples was 2−10. The subsamples (0.5−2.0 kg) were diced when necessary, and identical amounts (usually 100 g) of each were added to the pool. The samples were analyzed as normally consumed, that is, only the edible parts were included in the pool and analyses. Prior to pooling, apples were peeled and the peeled fruits and peels were analyzed separately. In the case of potato, boiled peeled tubers, boiled peels, and raw peels were pooled for analysis. Most of the fruit and vegetable samples were freeze-dried. The pooled fresh and freeze-dried samples were frozen and stored at −20 °C until analyzed.

Standards and Solvents. The mixture of six anthocyanidin glycosides, representing 3-*O*-glucosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, was obtained from Polyphenols Laboratories AS (Sandnes, Norway), and ellagic acid was obtained from Sigma Chemical Co. (St. Louis, MO). Acetonitrile (HPLC grade), methanol (HPLC grade), formic acid, and hydrochloric acid (HCl) were purchased from VWR International Ltd. (Espoo, Finland).

Analysis of Anthocyanins. Frozen samples were thawed and homogenized with a household blender, and four replicate samples (0.5−2 g of freeze-dried or 4−6 g of fresh sample) were weighed for the analysis. On the basis of our previous experimental knowledge (*25*), an acidified methanol was used for an extraction of anthocyanins. An optimized anthocyanin yield was achieved at room temperature with a three-step procedure. In the first and second steps, 80% aqueous methanol (15 mL) containing 2% HCl was added into the homogenate. After vigorous mixing for 1 min, the suspension was centrifuged at 3500*g* for 10 min, and the supernatant was removed. In the third step, the sediment was extracted with 50% methanol (15 mL) containing 1% HCl followed by mixing, centrifugation, and removal of supernatant. All three supernatants were combined and made up to 50 mL with purified water. The pH of the final samples was below 1.

Prior to HPLC analysis, the samples were filtered through a 0.45 μm syringe filter (Pall Life Sciences, Ann Arbor, MI). A 10 or 20 μL injection of the filtrates was separated on a 250 × 4.6 mm i.d., 5 μm LiChroCart Purospher Star RP-18e column (Merck, Darmstadt, Germany) with a 4 × 4 mm i.d. guard column using an HP 1100 series HPLC (Waldbronn Analytical Division, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a diode array detector linked to an HP ChemStation data handling system. The analysis of anthocyanins was performed using 8.5% formic acid in water as eluent A and HPLC grade acetonitrile/methanol (85:15, v/v) as eluent B. Two gradient elution programs were needed to get sufficient separation of all anthocyanins present in the samples. All of the berry and some fruit samples containing mainly nonacylated anthocyanins were analyzed with a gradient program as follows: 0−4 min, from 4 to 6% B; 4−6 min, from 6 to 7% B; 6−14 min, 7% B; 14−20 min, from 7 to 9% B; 20−30 min, from 9 to 10% B; 30−52 min, 10% B; 52−55 min, from 10 to 12% B; 55−65 min, from 12 to 15% B; 65−77 min, from 15 to 27% B; 77−81 min, from 27 to 36% B; 81−84 min, from 36 to 80% B, followed by an isocratic elution for 4 min and then return to the initial conditions for 5 min before the next injection. The flow rate of the mobile phase was 1.0 mL/min for 0−13 min and 0.8 mL for 14−90 min. The gradient program used for vegetables and fruits containing acylated anthocyanins was as follows: 0−3 min, from 4 to 9% B; 3−6 min, from 9 to 16% B; 6−15 min, 16% B; 15−19 min, from 16 to 20% B; 19−30 min, 20% B; 30−34 min, from 20 to 22% B; 34−48 min, from 22 to 30% B; 48−50 min, from 30 to 80% B, followed by an isocratic elution for 3 min and then return to the initial conditions for 5 min before the next injection. The flow rate of the mobile phase was 1.0 mL/min for 0−15 min and 0.8 mL for 15−55 min. Anthocyanins were detected at 520 nm. Because most of the anthocyanins present in the studied samples have been previously published and identified using HPLC-ESI-MS by both our own and other laboratories (*13*, *26*−*29*), no mass spectrometric analyses were performed in this study. Reference compounds and retention times and UV−vis spectra provided by HPLC-DAD were used to verify the identity of anthocyanins. Quantification was carried out using relevant anthocyanidin 3-*O*-glucosides as external standards. This means that all of the cyanidin derivatives were quantified with cyanidin 3-*O*-glucoside, all of the delphinidin derivatives with delphinidin 3-*O*-glucoside, etc. The contents were expressed in milligrams per 100 g of sample fresh weight, for the weight of the aglycone, and were calculated according to the following equation: $c_s = \sum (M_q A_q c_{std})/(M_{std} A_{std})$, where c_s = anthocyanidin concentration in the sample, M_q = molecular weight of the aglycone of the anthocyanin to be quantified, A_q = chromatographic peak area of the anthocyanin to be quantified, c_{std} = anthocyanin concentration of the relevant standard (anthocyanidin 3-*O*-glucoside),

Table 1. Foods Screened for the Presence of Anthocyanins and Ellagitannins[a]

berries	fruits	vegetables	nuts	processed foods
bilberry (wild)$^{A+,E-}$	apple, Granny Smith^{A-}	eggplant^{A+}	peanut^{E-}	bilberry soup^{A+}
black currant$^{A+,E-}$	apple, Red Delicious^{A+}	lettuce, Lollo rosso$^{A+,E-}$		cider, apple^{A-}
blueberry (cultivated)$^{A+,E-}$	apple, Valkea kuulas^{A-}	paprika, red$^{A-,E-}$		cider, pear^{A-}
bog whortleberry$^{A+,E-}$	grapefruit, red^{A+}	potato, Rosamunda^{A+}		mixed berry juice^{A+}
chokeberry$^{A+,E-}$	orange, red^{A-}	red beet^{A-}		raspberry jam$^{A+,E+}$
cloudberry$^{A+,E+}$	cherry$^{A+,E-}$	red cabbage$^{A+,E-}$		red cabbage salad^{A+}
cranberry$^{A+,E-}$	grape, red$^{A+,E-}$	red onion^{A+}		red wine^{A+}
crowberry$^{A+,E-}$	grape, green$^{A-,E-}$	red radish^{A+}		strawberry jam$^{A+,E+}$
gooseberry, red$^{A+,E-}$	nectarine$^{A+,E-}$	rhubarb^{A+}		
gooseberry, yellow$^{A-,E-}$	peach$^{A+,E-}$	tomato^{A-}		
lingonberry$^{A+,E-}$	pear$^{A-,E-}$			
raspberry$^{A+,E+}$	plum, dark$^{A+,E-}$			
red currant$^{A+,E-}$	watermelon^{A-}			
rose hip$^{A+,E+}$				
rowanberry$^{A+,E-}$				
saskatoon berry$^{A+,E-}$				
sea buckthorn$^{A-,E+}$				
strawberry, Honeoye$^{A+,E+}$				
strawberry, Jonsok$^{A+,E+}$				
strawberry, Polka$^{A+,E+}$				
sweet rowanberry, Burka^{A+}				
sweet rowanberry, Eliit^{A+}				
sweet rowanberry, Granatnaja^{A+}				

[a] Key: A, anthocyanin; E, ellagitannin; +, was detected; −, was not detected.

M_{std} = molecular weight of the relevant standard, and A_{std} = chromatographic peak area of the relevant standard.

The linearity of detector response was tested using the anthocyanin standard mixture in the concentration range of 4.0−240 μg/mL for individual anthocyanins and was found to be acceptable ($R^2 > 0.998$). The working solutions of the anthocyanin standard mixture were diluted from the 1.0 mg/mL stock solution in methanol. Freeze-dried bilberry was used as an "in-house" reference sample for determining the injection and interbatch (day to day) precisions of the anthocyanin analysis. To determine the injection precision, the reference sample was analyzed 14 times in the same operating conditions over one day. Interbatch precision was determined by analyzing the reference sample monthly during a 13 month period. Coefficients of variation (CV) for the injection and interbatch precisions were 0.7 and 7.0%, respectively. These values were considered to be acceptable.

Analysis of Ellagitannins and Ellagic Acids. Ellagitannins were determined as ellagic acid equivalents after acid hydrolysis using a slightly modified method optimized by Häkkinen and co-workers (*6*). Frozen samples were thawed and homogenized with a household blender. Four replicate 1.5−6 g samples of the homogenate were weighed, and 20 mL of methanol, 5 mL of water, and 5 mL of concentrated HCl were added. The solution was refluxed for 20 h at 85 °C. After hydrolysis, the solution was cooled at room temperature and made up to 50 mL with methanol. Free ellagic acid and its glycosides were analyzed without acid hydrolysis. The extraction was performed with 15 mL of 80% methanol. After vigorous mixing for 1 min, the suspension was centrifuged at 3500*g* for 10 min. The extraction procedure was repeated three times, and the supernatants were combined and made up to 50 mL with methanol.

Prior to HPLC analysis, the samples were filtered through a 0.45 μm syringe filter, and a 10 or 20 μL injection of the filtrates was separated on a 125 × 3 mm i.d., 5 μm LiChroCart Purospher RP-18e column (Merck, Darmstadt, Germany) with a 4 × 4 mm i.d. guard column using the HPLC equipment described above. The mobile phase consisted of aqueous 1% formic acid (eluent A) and acetonitrile/methanol (85:15, v/v) (eluent B). Ellagic acid was analyzed with gradient elution as follows: 0−20 min, from 5 to 30% B; 20−30 min, from 30 to 90% B, followed by an isocratic elution for 5 min and then return to the initial conditions for 5 min before the next injection. The flow rate was 0.5 mL/min. Ellagic acid and its derivatives were detected at 254 nm and quantified as ellagic acid equivalents. Identification of ellagic acid and its derivatives was based on ellagic acid standard using UV−vis spectra and retention time, literature data (*7*), and our previous studies (*13*).

The stock solution of ellagic acid standard (1.0 mg/mL) was prepared by first dissolving it in one part of dimethyl sulfoxide followed by four parts of methanol. The working solutions (0.01−0.4 mg/mL) were diluted from the stock solutions with methanol. The stock and working solutions were prepared at the time of analysis. The linearity of detector response for ellagic acid was acceptable ($R^2 > 0.998$). The CV of the injection precision was 0.3% and was determined using five injections of acid-hydrolyzed raspberry sample over one day. In addition, the effect of acid hydrolysis on pure ellagic acid was tested with a standard solution (0.05 mg/mL) and a spiked raspberry sample (1.5 mg of ellagic acid added). No decomposition of ellagic acid was observed.

Statistics. To study the differences between cultivars and harvesting seasons of strawberry, one-way analysis of variances (ANOVA) with Dunnett's C post hoc test was used for statistical analysis. The annual variation of raspberry and bilberry was examined by using Student's *t* test. All data were processed by SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Differences at $p < 0.05$ were considered to be significant.

RESULTS

Composite samples of 54 food items were analyzed for the presence of anthocyanins and 33 food items for ellagitannins (**Table 1**). For strawberries, sweet rowanberries, and apples, three cultivars were studied separately. In addition, we analyzed berry samples representing different harvest years. The quantitative analyses were performed using four replicates of the composite samples. The repeatability of the methods was assessed by determining the CV values for the replicate analyses. The CV values of the anthocyanin analyses were below 6% for berry samples and 1−17% for fruit and vegetable samples, and those for ellagitannin analyses were 2−17%. These variations were considered to be acceptable for repeatable and precise quantification of anthocyanins and ellagitannins.

Anthocyanin Content of Foods. The contents and distribution of individual aglycones (anthocyanidins) in berries, fruits, vegetables, and processed products are presented in **Tables 2−4**. Anthocyanins were detected in 41 food items, that is, in 21 of 23 berries, in 7 of 13 fruits, in 7 of 10 vegetables, and in 6 of 8 processed products. All of the six common anthocyanidins (cyanidin, delphinidin, malvidin, petunidin, pelargonidin, and peonidin) were detected in the foods studied. Cyanidin was the most common anthocyanidin and was always present whenever

Table 2. Contents of Anthocyanins in Fresh and Processed Berries[a,b]

sample	Latin name	year	mg/100 g of fresh weight						
			Cy	Dp	Pg	Pn	Pt	Mv	total
bilberry	*Vaccinium myrtillus*	2003	181.2	217.6		36.1	79.1	97.3	611.3
		2005	173.8	178.9		31.5	63.6	68.5	516.3
bilberry soup		2005	12.4	16.3		2.0	5.6	6.1	42.4
black currant	*Ribes nigrum*	2003	99.3	101.7					201.0
blueberry	*Vaccinium* spp.	2004	37.9	76.8		5.0	31.9	66.9	218.5
bog whortleberry	*Vaccinium uliginosum*	2004	9.0	46.6		5.1	23.4	69.9	154.0
chokeberry	*Aronia medik*	2004	410.2						410.2
cloudberry	*Rubus chamaemorus*	2004	1.0						1.0
cranberry	*Vaccinium oxycoccus*	2003	31.3	0.9		31.0	1.0	2.5	66.7
crowberry	*Empetrum nigrum*	2004	77.5	118.3		22.0	42.1	99.7	359.6
gooseberry, red	*Ribes uva-crispa*	2004	32.3						32.3
lingonberry	*Vaccinium vitis-idaea*	2003	76.9			0.6			77.5
mixed berry juice		2004	0.5	0.6				0.1	1.2
raspberry	*Rubus idaeus*	2003	38.5		0.9				39.4
		2005	53.9						53.9
raspberry jam		2004	5.7		0.2				5.9
red currant	*Ribes rubrum*	2003	21.7						21.7
rose hip	*Rosa rugosa*	2004	1.4						1.4
rowanberry	*Sorbus aucuparia*	2004	13.6						13.6
saskatoon berry	*Amelanchiar alnifolia*	2004	234.3						234.3
strawberry, Honeoye	*Fragaria* × *ananassa*	2004	1.3		36.0				37.3
strawberry, Jonsok	*Fragaria* × *ananassa*	2003	1.4		51.0				52.4
		2004	1.2		34.5				35.7
strawberry, Polka	*Fragaria* × *ananassa*	2003	1.7		30.9				32.6
		2004	2.8		29.4				32.4
		2005	5.0		44.8				49.8
strawberry jam		2004	0.3		2.6				2.9
sweet rowanberry, Burka	*Sorbus aucuparia* × [*Sorbus aria* (L.) Crantz × *Aronia arbutifolia* (L.) Pers.]	2005	168.1						168.1
sweet rowanberry, Eliit	*Sorbus aucuparia* × *Pyrus* sp. × *Sorbus aucuparia* var. Moravica	2005	24.9						24.9
sweet rowanberry, Granatnaja	*Sorbus aucuparia* × *Crataegus sanguinea* Pallas	2005	86.7						86.7

[a] Contents of individual and total anthocyanins are expressed as the weight of the aglycone moieties and presented as means of four replicates. The CVs of the replicate values were ≤6%. [b] Abbreviations: Cy, cyanidin; Dp, delphinidin; Pg, pelargonidin; Pn, peonidin; Pt, petunidin; Mv, malvidin; total, sum of the individual aglycones.

Table 3. Contents of Anthocyanins in Fruits[a,b]

sample	Latin name	year	mg/100 g of fresh weight						
			Cy	Dp	Pg	Pn	Pt	Mv	total
apple, Red Delicious	*Malus domestica*	2004	1.7						1.7
apple peel, Red Delicious		2004	13.2[c]						13.2
grapefruit, red	*Citrus maxima*	2005	5.9						5.9
cherry	*Prunus avium*	2005	64.4			2.0			66.4
grape, red	*Vitis vinifera*	2005	11.8	0.7		3.6	1.0	20.5	37.6
red wine		2004	12.8[c]						12.8
nectarine	*Prunus persica* var. *nectarina*	2005	2.4						2.4
peach	*Prunus persica*	2004	4.2						4.2
plum, dark	*Prunus domestica*	2005	25.1						25.1

[a,b] See **Table 2**. The CVs of the replicate values were 3–17%. [c] Contains unidentified acylated anthocyanins, and thus all of the compounds are quantified as cyanidin.

anthocyanins were detected, except in eggplant and red radish. All six aglyconic forms were found in berries, whereas cherry, red grape, and red radish were the only foods among fruits and vegetables containing aglycones other than cyanidin. Delphinidin, petunidin, peonidin, and malvidin were found in red grape. Pelargonidin was detected in red radish, and peonidin was present in cherry. With the exception of blueberry and strawberry, only nonacylated anthocyanidin glycosides were found in berries. The most diverse profile of anthocyanins was detected in bilberry (**Figure 2**), blueberry, and bog whortleberry. Acylated anthocyanins were present in blueberry, strawberry, apple, red grape, red grapefruit, and most of the vegetables.

The total anthocyanin content varied in berries from 1 to 611 mg/100 g, in fruits from 2 to 66 mg/100 g, and in vegetables from 3 to 75 mg/100 g of fresh weight. The highest contents were found in bilberry, chokeberry, and crowberry (**Table 2**). In fruits, the highest levels were detected in cherry, red grape, and dark plum (**Table 3**). Among vegetables, red cabbage, red radish, and red onion had the highest contents (**Table 4**). In apple and potato, the anthocyanins detected were almost exclusively located in the peels, and only minor levels were found in the peeled apple fruits. The anthocyanin content found in processed foods was much lower than in the corresponding raw materials. In commercially available berry soup and jams and red cabbage salad, the anthocyanin concentrations were 7−12% of those found in the berries and cabbage. The anthocyanin quantities present in processed foods were determined as in the sample; that is, the results were not corrected for either the effects of processing or the percentage of raw material used in manufacture.

The annual variations in the anthocyanin content of some berries were investigated, and significant differences between

Table 4. Contents of Anthocyanins in Vegetables[a,b]

sample	Latin name	year	mg/100 g of fresh weight						
			Cy	Dp	Pg	Pn	Pt	Mv	total
eggplant	*Solanum melongena*	2004		7.5					7.5
lettuce, Lollo rosso	*Lactuca sativa* var. *crispa*	2005	5.2						5.2
potato, Rosamunda, cooked, peeled		2004							nd[c]
potato peel, Rosamunda, cooked		2004	3.5[d]						3.5
potato peel, Rosamunda, raw		2004	6.5[d]						6.5
red radish	*Raphanus sativus*	2003			32.0				32.0
red cabbage	*Brassica olerace* var. *capitata* "*f. rubra*"	2003	75.1						75.1
red cabbage salad		2005	6.1						6.1
red onion	*Allium cepa* var. *cepa*	2003	15.1						15.1
rhubarb	*Rheum rhabarbarum*	2005	3.9						3.9

[a,b] See **Table 2**. The CVs of the replicate values were 1–15%. [c] Below detection limit. [d] Contains unidentified acylated anthocyanins, and thus all of the compounds are quantified as cyanidin.

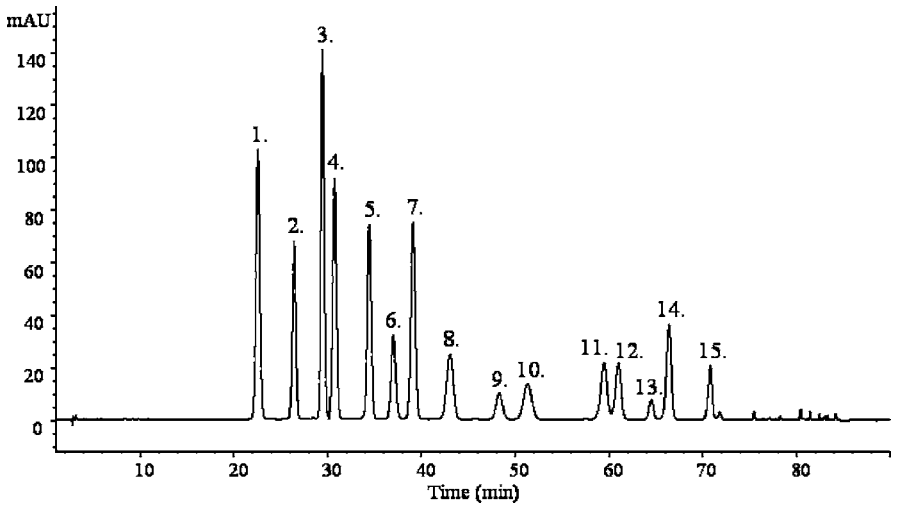

Figure 2. HPLC chromatogram at 520 nm of anthocyanins in bilberry. Peaks: 1, delphinidin 3-galactoside; 2, delphinidin 3-glucoside; 3, cyanidin 3-galactoside; 4, delphinidin 3-arabinoside; 5, cyanidin 3-glucoside; 6, petunidin 3-galactoside; 7, cyanidin 3-arabinoside; 8, petunidin 3-glucoside; 9, peonidin 3-galactoside; 10, petunidin 3-arabinoside; 11, peonidin 3-glucoside; 12, malvidin 3-galactoside; 13, peonidin 3-arabinoside; 14, malvidin 3-glucoside; 15, malvidin 3-arabinoside.

harvesting seasons were observed. The anthocyanin contents in bilberry and raspberry (collected in the summers of 2003 and 2005) were 611 ± 7.3 and 516 ± 25.8 mg/100 g ($p < 0.001$) and 39 ± 0.5 and 54 ± 0.6 mg/100 g ($p < 0.001$), respectively. In strawberry cultivar Jonsok (harvested in the summers of 2003 and 2004) they were 52 ± 0.7 and 36 ± 3.3 mg/100 g ($p < 0.001$), and in cultivar Polka (harvested in the summers of 2003, 2004, and 2005) they were from 32 ± 1.3 to 50 ± 0.4 mg/100 g ($p < 0.001$) (**Table 2**). In addition, some differences in the anthocyanin content between three strawberry cultivars (harvested in the summer of 2004) were detected. The anthocyanin content present in cultivar Honeoye (37 ± 0.4 mg/100 g) was significantly higher ($p < 0.001$) than that of cultivar Polka (32 ± 0.3 mg/100 g), whereas no significant differences were observed between cultivars Jonsok and Polka or Honeoye.

Ellagitannin Content of Foods. Ellagic acid was found in 9 of 33 selected food items (**Table 5**), after acid hydrolysis of the samples. Three individual compounds with similar UV−vis spectra were taken into account in the quantification. These compounds were considered as the conversion products of ellagitannins representing ellagic acid and its two derivatives (**Figure 3**). The total ellagic acid content varied from 1 to 330 mg/100 g of fresh weight. Ellagic acid was mostly present as ellagitannins, and the relative amount of free ellagic acid and its glycosides (non-tannin ellagic acid) was <6%, and in most cases only 1−2%.

Berries of the family Rosaceae (cloudberry, raspberry, rose hip, and strawberry) contained high levels of ellagic acid equivalents, whereas minor levels were found in sea buckthorn (family Elaeagnaceae). Ellagic acid compounds were detected only in these five berries. The total ellagic acid contents in raspberry and strawberry jams were 23−36% of those found in the unprocessed berries. The relative amount of free ellagic acid compounds was as high as 11% in strawberry jam, in contrast to 3% in raspberry jam. This may suggest that during strawberry processing ellagitannins were decomposed and converted to ellagic acid more easily than during raspberry processing. All berries and jams were analyzed "as is" without separation of the seeds from the food matrix.

The total ellagic acid contents in different strawberry cultivars and different harvesting seasons varied from 68.3 to 85.3 mg/100 g. No statistically significant differences were observed between cultivars or harvesting seasons.

DISCUSSION

In a recent study (*2*), the anthocyanin contents in common foods in the United States were investigated. These results were published during the preparation of this paper. Wu and coworkers (*2*) reported that anthocyanins were found in 24 of over 100 foods screened and that the highest levels were detected in chokeberry, elderberry, blueberry, and black currant. In the

Table 5. Contents of Ellagitannins and Free Ellagic Acid Derivatives in Foods[a]

sample	Latin name	year	mg/100 g of fresh weight		
			ellagitannins	free ellagic acid derivatives	total
cloudberry	*Rubus chamaemorus*	2004	311.8	3.3	315.1
raspberry	*Rubus idaeus*	2003	260.0	3.7	263.7
		2005	326.2	4.7	330.9
raspberry jam		2004	74.1	2.3	76.4
rose hip	*Rosa rugosa*	2004	107.5	2.1	109.6
sea buckthorn	*Hippophae rhamnoides*	2003	1.0	nd[b]	1.0
strawberry, Honeoye	*Fragaria* × *ananassa*	2004	75.4	2.2	77.6
strawberry, Jonsok	*Fragaria* × *ananassa*	2003	78.5	1.4	79.9
		2004	71.4	4.3	75.7
strawberry, Polka	*Fragaria* × *ananassa*	2003	67.6	0.7	68.3
		2004	83.2	2.1	85.3
		2005	73.8	1.9	75.7
strawberry jam		2004	21.7	2.8	24.5

[a] Contents are expressed as ellagic acid equivalents and presented as means of four replicates. The CVs of the replicate values were 2–17%. [b] Below detection limit.

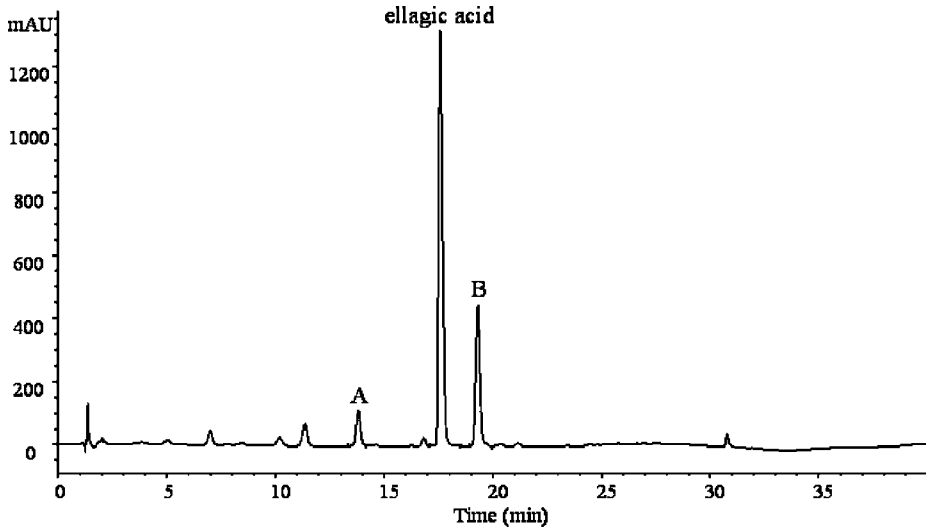

Figure 3. HPLC chromatogram at 254 nm of raspberry after acid hydrolysis. Peaks: 1, ellagic acid derivative A; 2, ellagic acid; 3, ellagic acid derivative B.

present study, the anthocyanin content in foods consumed in Finland was determined. Selection of the foods was mainly based on the color of food, that is, blue, purple, red, and orange colors were considered to be a positive indicator for the potential presence of anthocyanins. Of the 54 food items, anthocyanins were detected in 41 food items representing 21 berries, 7 fruits, and 7 vegetables. The present results and the study reported by Wu and co-workers (*2*) support the fact that berries are a superior source of anthocyanins. The 20 best sources of anthocyanins in the Finnish diet include 15 berries or berry products, 3 fruits, and 2 vegetables.

Our results show that dark blue/red berries contain anthocyanins at >150 mg/100 g, whereas the content in fruits, vegetables, and red/orange berries is <100 mg/100 g. The anthocyanin contents in common foods in the United States (*2*) were systematically higher than those in this study. However, these results are not comparable as such due to the different quantification methods. The anthocyanin contents in the present study are expressed as the weight of the aglycones (anthocyanidins) of the anthocyanins to be quantified, whereas the results reported by Wu and co-workers (*2*) were expressed for the weight of the anthocyanins (anthocyanidin glycosides) or acyl derivatives of the anthocyanins. Comparison of the anthocyanin quantities between several published studies is difficult and inaccurate for the reason mentioned above or for the following reasons. Different analytical methods (spectrophotometric or HPLC) can be used for quantitative analysis of anthocyanins, or the results may be calculated by using one standard (cyanidin 3-glucoside) only or a mixture of relevant anthocyanidin 3-glucosides. The major differences in anthocyanin quantities may be derived from the different calculation methods used. It is extremely important to express the calculation method used in each study, because the quantification is based on molecular weights of both the anthocyanin to be quantified and the standard anthocyanin. In this study, the quantification of anthocyanins was based on six relevant anthocyanidin 3-glucoside standards and the calculations were performed as mentioned above. As we have reported our results as anthocyanidins (aglycones), our data are comparable to those published in the USDA Database for the Flavonoid Content of Selected Foods 2006 (available at http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02.pdf).

The quantitative and qualitative analyses of anthocyanins are not possible with insufficient analytical methods. In this study, HPLC with two different gradient programs was used to get a good separation of all anthocyanins present in the samples. For nonacylated anthocyanins, the separation of all individual anthocyanins was achieved with a slow gradient elution. The retention of acylated anthocyanins, however, was higher than those of nonacylated compounds, resulting in an insufficient

separation with this method. Therefore, a more rapid gradient elution was needed to decrease the retention and to improve the separation of individual acylated compounds.

Retention times and UV−vis spectra are useful and important tools for the qualitative analysis of anthocyanins. As Wu and Prior (*26*) noted, the elution for some common anthocyanidin glycosides using reverse-phase HPLC seems to fit most experimental conditions. By means of the UV−vis spectra of anthocyanins the structures of aglycone moieties and acylated and nonacylated anthocyanins can be distinguished. From our experience, however, the UV−vis spectra of (a) delphinidin, malvidin, and petunidin and (b) cyanidin and peonidin resemble each other, which can reduce the reliability of structural information. In addition, the elution order (retention times) for complex anthocyanins is mostly dependent on the substitution pattern (sugar and acyl moieties) and may differ from that of common anthocyanins. HPLC coupled with mass spectrometry offers a powerful method for more precise identification of anthocyanins than retention times and UV−vis spectra by providing exact information about the molecular weight and fragmentation of compounds. Furthermore, the coeluting peaks, which are common in samples with complex anthocyanin compositions, can be distinguished by means of MS data, if the fragmentation patterns of the molecules differ (*26*). However, MS data will not necessarily provide all of the information about the anthocyanin molecule; for example, the structures of sugar and acyl moieties may not be completely identified.

According to previous studies (*30*−*32*), the ellagitannin contents of raspberry and strawberry were 160 and 25−59 mg/100 g in fresh samples, respectively. These contents are lower than in the present study. The explanation for this is that in the previous studies ellagitannins were quantified as one conversion product after acid hydrolysis. In our previous study (*13*) we noted that ellagitannins were converted to ellagic acid and a less polar derivative after acid hydrolysis. Vrhovsek and co-workers (*7*) showed that besides ellagic acid, two ellagic acid derivatives were detected in raspberry samples after acid hydrolysis of ellagitannins. This is supported by our present findings, which also indicate that these derivatives are present whenever acid hydrolysis is used for determining the ellagitannins, and thus the quantification should be based on three conversion products instead of one.

In previous studies (*33*, *34*) the leaves of rose hip (*Rosa rugosa*) and sea buckthorn (*Hippophae rhamnoides*) have been reported to contain ellagitannins. To our knowledge, however, this was the first time when ellagitannins were detected in berries of rose hip and sea buckthorn plants.

For estimation of the mean degree of ellagitannin polymerization (mDEP) in the present samples, the model published by Vrhovsek and co-workers (*7*) was used. This estimate provides valuable information on the ratio of non-tannin and tannin ellagic acids as well as on the structures of simple and oligomeric ellagitannins. In the present study, ellagic acid and its derivatives were quantified without any corrections in molar absorptivities. Therefore, the theoretical mDEP is derived from the ratio of the concentrations of ellagic acid and its derivative (derivative B in **Figure 3**) as ellagic acid equivalent produced in the acid hydrolysis. The theoretical mDEP can be calculated from the following equation: $\text{mDEP} = (\text{R[EAdB]/[EA]} + 1)/(1 - \text{R[EAdB]/[EA]})$, where R[EAdB]/[EA] refers to the ratio of the concentrations of ellagic acid (EA) and its derivative (EAdB) (*7*). As shown in **Table 6**, among cloudberry, raspberry, rose hip, and strawberry the mDEP differs considerably. These values suggest that oligomeric ellagitannins are more abundant in strawberry, whereas monomers are predominant in rose hip. In cloudberry the oligomers with DP > 2 roughly equal the monomers in absolute concentration as well as in raspberry, as was noted by Vrhovsek and co-workers (*7*). However, the ratio between ellagic acid and its derivatives after acid hydrolysis is strongly dependent on the ellagitannin structures present in foods. Thus, these mDEP values are only estimates and may be misleading, if the structural information of ellagitannins is invalid.

Table 6. Estimation of the Mean Degree of Ellagitannin Polymerization (mDEP) in Cloudberry, Rose Hip, Raspberry, and Strawberry

sample	year	ratio [EAdB]/[EA][a]	mDEP
cloudberry	2004	0.33	2.0
raspberry	2003	0.36	2.1
	2005	0.36	2.1
rose hip	2004	0.24	1.6
strawberry, Honeoye	2004	0.43	2.5
strawberry, Jonsok	2003	0.39	2.3
	2004	0.41	2.4
strawberry, Polka	2003	0.41	2.4
	2004	0.40	2.3
	2005	0.39	2.3

[a] Ratio of the concentrations of ellagic acid derivative B (EAdB) and ellagic acid (EA) determined as ellagic acid equivalents after acid hydrolysis.

In conclusion, in foods consumed in Finland anthocyanins are primarily found in berries and berry products, and the highest levels are present in dark blue or red species. Ellagitannins are almost exclusively present in certain berries of the family Rosaceae, and not in fruits and vegetables. The present results will be entered into the Finnish Food Composition Database Fineli (available at http://www.fineli.fi), maintained by the National Public Health Institute of Finland. On the basis of this data, daily intake estimates of anthocyanins and ellagitannins from the Finnish diet will be calculated.

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